

Polymorphic CYP2A6 and its clinical and toxicological significance

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The cytochrome P450 (CYP) enzymes play a crucial role in the metabolism of exogenous compounds including pharmaceutical agents. It is estimated that about one-half of drugs are primarily cleared from the body through the action of CYP enzymes mainly in the liver.¹ About 40% of human CYP-dependent drug metabolism is carried out by polymorphic enzymes, which can result in therapeutic failures and adverse effects from ensuing interindividual variability in drug concentrations. Several examples exist where subjects carrying certain variant alleles suffer from adverse effects from drug treatment due to the presence of defective alleles.² The well-known polymorphic CYP genes are *CYP2C9*, *CYP2C19* and *CYP2D6*. Many of the polymorphic CYP enzymes also metabolize numerous toxicologically significant compounds, such as food additives, pollutants, solvents, and recreational drugs, many of which cause acute or delayed toxic effects.³

CYP2A6 represents up to 10% of human microsomal P450 proteins. CYP2A6 is the major enzyme catalysing the oxidative metabolism of nicotine and cotinine and it contributes to the metabolism of some pharmaceuticals (eg fadrozole, tegafur, SM-12502), nitrosamines, other carcinogens (eg aflatoxin B1) and a number of coumarin-type alkaloids.^{4–6} CYP2A6 may be inducible by antiepileptic drugs and it is decreased in alcohol-induced severe cirrhosis.⁴

The members in human CYP2A gene subfamily are *CYP2A6*, *CYP2A7* and

CYP2A13.⁷ CYP2A6 is expressed in the liver whereas CYP2A13 is an extrahepatic enzyme; the *CYP2A7* gene encodes an unstable and inactive protein.⁵ The organization and structure of the *CYP2A* gene cluster and several polymorphic alleles of the *CYP2A6* gene have now been characterized (see <http://www.imm.ki.se/CYPalleles>). There are three gene deletion alleles (*CYP2A*4A*, **4B* and **4D*) and numerous SNPs. Recently, in *Pharmacogenetics* journal a paper by Ariyoshi *et al.*⁸ was published concerning the gene deletion allele *CYP2A6*4B* in Japanese. During their previous studies⁹ the group had already identified two genotypes of the *CYP2A6* gene (D-type and E-type or *CYP2A6*4A*; see <http://www.imm.ki.se/CYPalleles>). These earlier results could be interpreted as the D-type deletion being a partial *CYP2A6* gene-deleted allele. However, studies on the *CYP2A6* gene have been rather problematic, because the highly (94%) homologous *CYP2A7* gene is located just 25 kb upstream of the *CYP2A6* gene. For example, the allele termed *CYP2A6*3*, which was one of the earliest variants found, is most probably an artefact based on the presence of *CYP2A7*-derived 3'-sequences in the *CYP2A6*1B* allele and the consequent amplification of *CYP2A7* sequences by the original PCR-based genotyping assay for *CYP2A6*1*, *CYP2A6*2* and *CYP2A6*3* alleles.¹⁰

Ariyoshi *et al.* showed in their paper that by combining different types of methods they could resolve the discrepant results in PCR, PCR-RFLP and Southern blot analyses when genotyping the samples of the same subjects.

The published study showed that the genotype previously named as 'D-type' is actually composed of *CYP2A6*4A* and an entire *CYP2A6* gene-deleted allele, *CYP2A6*4B*. They estimated that the allele frequency of the *CYP2A6*4B* in Japanese is 0.60% and that of the *CYP2A6*4A* 19.0%.

It is of interest that a significant proportion of the Japanese lack the CYP2A6 protein completely due to the relatively high incidence of *CYP2A6* gene deletion alleles in Japanese and Chinese populations.^{9,11} The frequency of poor metabolizers (PMs) in European and Middle East populations is about 1% or less, whereas it is much higher in the Asian populations (up to 20%). Also some alleles which decrease the ability of CYP2A6 to metabolize nicotine and coumarin have been found in higher frequencies in Japanese and Chinese in comparison with Caucasians.^{12,13}

Why is it of importance to elucidate the exact alleles and genotypes of *CYP2A6*? The main reasons to do this are the marked role of CYP2A6 in the metabolism of various substrates, especially pharmacologically and toxicologically relevant compounds, and the importance of complete and unequivocal elucidation of all functional alleles for global genotyping studies.

Interest in CYP2A6 has risen considerably after nicotine and some tobacco-specific nitrosamines were established as high-affinity substrates for this enzyme. Rao *et al.*¹⁴ reported that the individuals who are nicotine-dependent and have defective *CYP2A6* alleles smoked fewer cigarettes and it is assumed that the deletion alleles may be protective regarding cancer by the decreased metabolic activation of procarcinogens found in tobacco smoke. Several case-control studies have addressed the relationship between CYP2A6 status and smoking habits as well as the role of CYP2A6 polymorphisms in lung cancer risk, but the results thus far have been inconclusive.⁵ However, one reason for these inconclusive results may have been that only a few alleles have been assayed.

There are now more than 10 different allelic variants known to cause abolished or decreased enzyme activity. The inability to take into account all the functional variant alleles in addition to some methodological problems as shown by the article of Arioyshi *et al.* are almost certainly behind the inconclusive and discrepant results in published studies. It is worth pointing out the importance of identifying the correct genotype and its functional consequences unequivocally before a relationship between CYP2A6 status and various cancer risks is studied.

What is the toxicological and clinical significance of polymorphisms of CYP2A6? CYP2A6 deletion alleles (CYP2A6*4A, CYP2A6*4B and CYP2A6*4D) are of great importance in studies aimed at correlating smoking behaviour, pre-carcinogen activation or drug metabolism with the CYP2A6 genotype, especially in Oriental populations. It is important to know if the different alleles are producing active enzyme able to metabolize drugs and other perhaps toxic or carcinogenic chemicals. In molecular epidemiological studies or in clinical trials a need to measure the genotype status of all functionally relevant alleles of important CYP genes has become increasingly more acute.

It is possible that in the future, when physicians prescribe medication to their patients, they will have information on the patient's CYP status. Will the CYP2A6 gene be tested together with the CYP2C9, CYP2C19 and CYP2D6 genes? It seems quite likely that CYP2A6 is included occasionally,

if it is a rate limiting metabolizing enzyme for a drug that has 'dangerous' effects. There are already a couple of examples in which CYP2A6 pharmacogenetics might be of importance to know. Daigo *et al.*⁶ found that when giving tegafur (an anticancer drug) to gastric cancer patients, one patient had four-fold higher plasma tegafur concentrations than the others. Tegafur is metabolized via CYP2A6 to 5-fluorouracil, which is the active drug. Those persons with a CYP2A6 poor metabolizer status could not produce high enough concentrations of the active metabolite to have a beneficial effect of the drug treatment. Another example is a platelet-activating factor receptor antagonist, SM-12502, which affects blood coagulation. In this case, a CYP2A6 PM patient might be very sensitive to the drug.

Also single nucleotide polymorphisms (SNPs) can be of importance in addition to deletions, because they can change the substrate selectivity and turnover of the CYP2A6 enzyme.¹³ In drug development, ethnic differences regarding CYP2A6 must be taken into consideration if the new chemical entity is significantly metabolized via this enzyme. However, CYP2A6 could be even more significant in tobacco-related behaviours and diseases, because of its role in nicotine and nitrosamine metabolism. Thus far the elucidation of the role of CYP2A6 polymorphisms in smoking habits and ill effects has led to inconclusive findings, but it is hoped that the more complete and functionally relevant analysis of CYP2A6 polymorphisms and variant alleles, such as the one in

Arioyshi's paper, would give more definite answers in the future.

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DUALITY OF INTEREST

None declared.

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